

Low Within Population Genetic Variation and High Among Population Differentiation in *Cyrtomium falcatum* (L.f.) C. Presl (Dryopteridaceae) in Southern Korea: Inference of Population-Establishment History

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ABSTRACT.—In the Korean Peninsula, the current distribution of the warm-temperate and subtropical vegetation (including many homosporous ferns) is limited to southern coastal areas. Paleoecological data suggest that during the Last Glacial Maximum this vegetation retreated to glacial refugia putatively located in southern Japan and/or southern China, followed by a post-glacial recolonization. Two broad scenarios of post-glacial recolonization could be hypothesized: extant Korean populations are derived from multiple source populations (i.e., from multiple refugia); alternatively, they originate from a single refugium. To test which of these scenarios is more likely, we surveyed patterns of genetic diversity in eight ($n = 307$) populations of *Cyrtomium falcatum* from southern Korea. We found extremely low levels of allozyme variation within populations coupled with high among-population differentiation. These data best support the second hypothesis, and indicate that the current genetic diversity may be a consequence of post-glacial long-distance dispersal events and subsequent founder effects. In addition, restricted gene flow among the discontinuous populations of *C. falcatum* in southern Korea has likely contributed to the high degree of among-population genetic differentiation. From a conservation perspective, several populations should be targeted for both *in situ* and *ex situ* conservation, as *C. falcatum* exhibits a high degree of divergence among populations.

KEY WORDS.—Dryopteridaceae, *Cyrtomium*, allozymes, conservation, founder effect, glacial refugia, homosporous fern, gametophytic selfing, population history, population structure

Genetic diversity patterns of plant species are shaped by interacting historical, biological, ecological, and demographic factors (Nevo *et al.*, 1984;

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Hamrick and Godt, 1989; Gray, 1996; Duminil *et al.*, 2007). From a historical viewpoint, the Quaternary glacial-interglacial oscillations played an increasingly recognized role in shaping the current distribution of plant species and thus, their contemporary levels and partitioning of genetic diversity within and among populations (Hewitt, 1999, 2000; Hu *et al.*, 2009). For example, populations/species that occurred in formerly glaciated regions usually show lower levels of genetic diversity than those from unglaciated areas (e.g., glacial refugia) through founder effects and bottlenecks as a result of multiple stepwise colonization events (Hewitt, 1996; Widmer and Lexer, 2001; Jiménez *et al.*, 2010). Thus, the patterns of genetic diversity maintained by the species (especially the spatial distribution of genotypes) are often used to infer the location of refuges and the post-glacial migration routes from these, and this has been particularly fruitful in Europe and North America (Soltis *et al.*, 2006; Weiss and Ferrand, 2007, Hu *et al.*, 2009; Hewitt, 2011).

Ferns have some life-history traits that are strikingly different from seed plants and that have potentially significant effects on patterns of population genetic variation. First, fern dispersal occurs via haploid spores. Second, their gametophytic generation is independent from the maternal sporophytes. Third, owing to their small size, fern spores tend to be dispersed much farther by wind compared to most seeds (Tryon, 1970, 1972), although this feature is analogous to the tiny, dust-like seeds of orchids (Arditti and Ghani, 2000). As in many seed plants, however, the majority of propagules fall around the immediate vicinity of the parent (Peck *et al.*, 1990). Fourth, since spermatozoids require transport in water, male gamete dispersal distance of ferns tends to be very limited (within a few centimeters; Peck *et al.*, 1990). Finally, in many homosporous ferns, in the absence of genetic load a single spore could produce a sporophyte via intragametophytic selfing (self-fertilization of a haploid gametophyte), enabling the successful colonization of new sites (Lloyd, 1974; Flinn, 2006; Edgington, 2007; Wubs *et al.*, 2010). Intragametophytic selfing results, in a single generation, in completely homozygous sporophytes (Klekowski, 1972; Vogel *et al.*, 1999a), a situation without analogue in seed plants. Thus, it has been suggested that genetically polymorphic populations could be attributed to the occurrence of multiple independent spore dispersal and establishment events over time, whereas genetically monomorphic homosporous fern populations are more likely to have arisen from single colonists (i.e., single spores; Pryor *et al.*, 2001).

Habitat specificity and recurrent gene flow of homosporous ferns should be regarded as factors determining the degree of population differentiation. Soltis *et al.* (1989) hypothesized that xeric or rock dwelling ferns would exhibit higher among-population differentiation than would ferns occurring in mesic habitats, due to limited gene flow among isolated rocky habitats. Since then, several population-genetics studies have supported this hypothesis (Pryor *et al.*, 2001 and references therein).

In Korea, many ferns are characteristic of the warm-temperate and subtropical vegetation, such as *Cyrtomium falcatum* (L.f.) C. Presl (Dryopteridaceae), a rock dwelling homosporous fern native to southern and eastern

Asia, which is taken here as a case study. This vegetation belt currently occurs in a narrow zone along the southeastern and southern coast (Yi, 2011). The few available pollen and spore records suggest, however, that this warm-temperate vegetation was likely absent from the Korean Peninsula during the Last Glacial Maximum (LGM, ca. 21,000 yr ago; e.g., Choi, 1998; Chung *et al.*, 2006; Chung, 2007; Chung *et al.*, 2010; Yi and Kim, 2010), a scenario consistent with regional vegetation reconstructions (e.g., Adams and Faure, 1997; Harrison *et al.*, 2001; Hope *et al.*, 2004; Prentice *et al.*, 2011). On the southern coast of Korea, the onset of the Holocene (ca. 11,000 years BP) and the accompanying climatic amelioration were marked by a sudden increase in abundance of ferns in the family Polypodiaceae, and an abrupt decline in herbaceous taxa, together with the expansion of cool temperate deciduous broad-leaved forests (Chung *et al.*, 2010). The first appearance after the LGM of broad-leaved evergreen vegetation in the Korean Peninsula was approximately 8,500 years ago (Chung, 2011) and somewhat earlier in Jeju Island (ca. 12,000–10,000 yr BP; Chung, 2007), which also coincided with a rise of fern spores, indicating warmer and more humid conditions (Chung *et al.*, 2010). These paleovegetation studies suggest that post-glacial colonization either from southern Japan (e.g., Kyushu; Fig. 1) or southern China, which harbored glacial refugia for warm-temperate vegetation (e.g., Hope *et al.*, 2004; Gotanda and Yasuda, 2008; López-Pujol *et al.*, 2011; Qiu *et al.*, 2011), would be much more plausible than persistence of warm-temperate and subtropical vegetation in Korean refugia during the Pleistocene glaciations.

Cyrtomium falcatum is an evergreen homosporous fern that usually grows on coastal rocky slopes in the warmer parts of south to northeastern Asia (India, Vietnam, eastern and southern China, Taiwan, southern Korea, and Japan; Iwatsuki, 1992). However, it has become naturalized in many parts of the world (including Hawaii, North America, Australia, western and southern Europe, Réunion Island, and South Africa) because it escaped from gardens (Roux, 2011). The species, 10–60 cm tall, has a short, erect rhizome, and thus, it is highly likely that proximally located individuals within populations are distinct genets. In southern Korea, *C. falcatum* usually grows on crevices in steep cliffs, rocks, and man-made vertically oriented stone walls near seashores, and thus, populations occur discontinuously. Chromosome numbers of $n = 41$ (diploid) or $n = 82$ (tetraploid) have been reported for *C. falcatum* in Japan (Iwatsuki, 1992).

Based on the life-history and ecological traits of homosporous ferns, together with the information available on the paleoecology of the Korean Peninsula, we hypothesize two broad scenarios for the origin of current populations of warm-temperate homosporous fern species in southern Korea. If contemporary populations were derived from multiple source populations (i.e., from multiple glacial refugia), presumably from southern Japan and/or southern China, we would expect high levels of within-population genetic variation as consequence of the admixture of genetically divergent lineages arriving from different refugia (i.e., the ‘melting pot’ effect that has been described for many European trees and shrubs; Petit *et al.*, 2003). Regarding among-population

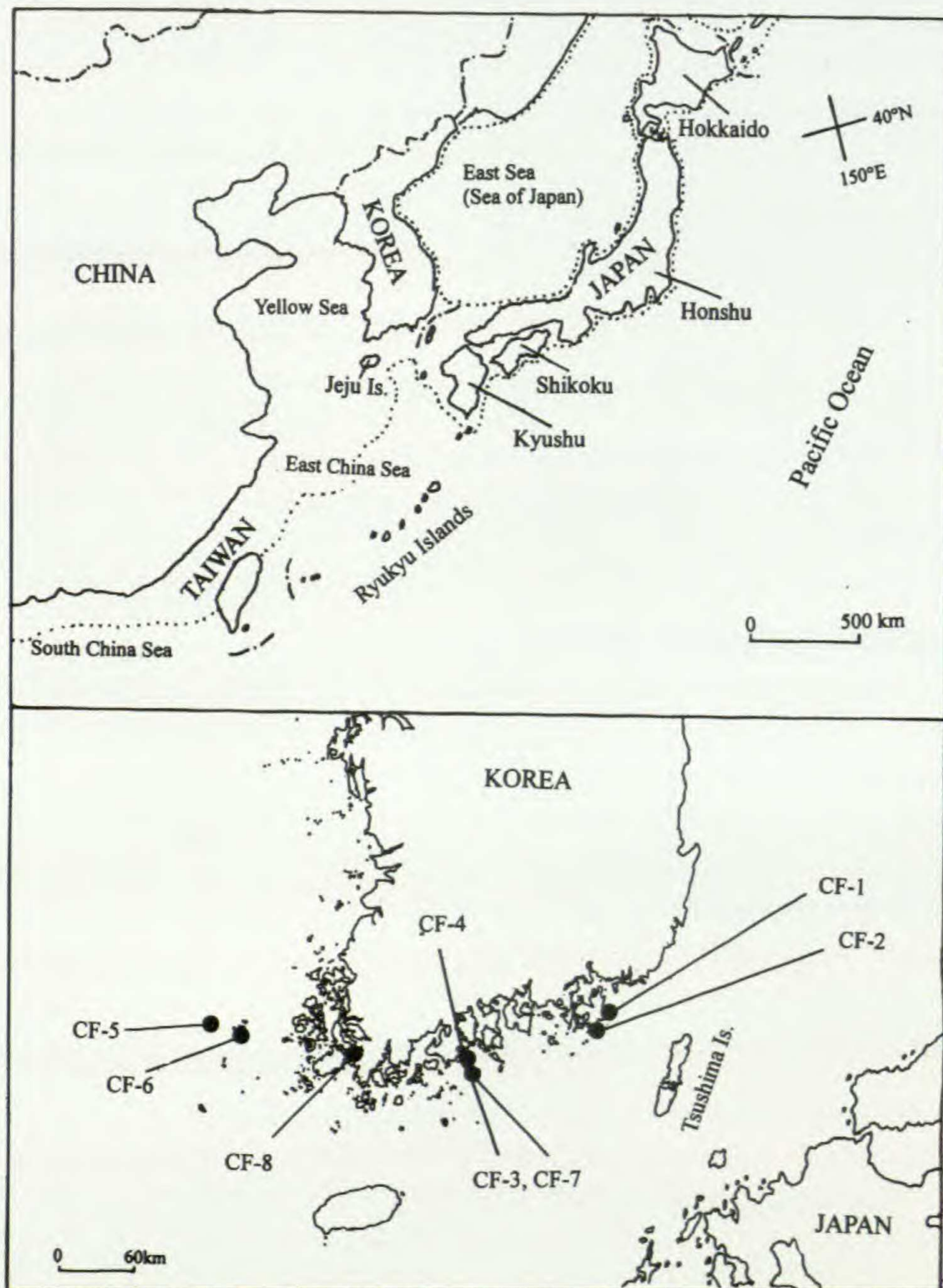


FIG. 1. Upper figure: map of northeastern Asia. Dotted line indicates exposed coastal lines during the Last Glacial Maximum (Shi, 2002; Xu *et al.*, 2010). Lower figure: collection sites of *Cyrtomium falcatum* in southern Korea; CF-1 and CF-2 in Geojae Island. CF-3 and CF-7 (each separated by ca. 1.5 km) in Oenaro Island; CF-4 in Naenaro Island; CF-5 in Hong Island; CF-6 in Heuksan Island; and CF-8 in Haenam-gun (mainland Korea).

genetic differentiation, either low or high values would be exhibited depending on ecological factors (Hamrick and Nason, 1996). Large populations that are continuously distributed should exhibit low inter-population variation probably due to high recurrent gene flow between adjacent populations. In contrast, high genetic divergence would be expected among small disjunct populations because of low rates of gene flow between isolated

TABLE 1. Summary of genetic diversity measures and mean fixation values (F_{IS}) observed in eight populations of *Cyrtomium falcatum*.

Population	n	% P	A	AR	H_o (SE)	H_e (SE)	F_{IS}
CF-1	36	14.3	1.14	1.14	0.044 (0.053)	0.043 (0.042)	-0.071
CF-2	36	14.3	1.14	1.12	0.039 (0.042)	0.046 (0.049)	0.154
CF-3	51	9.5	1.10	1.08	0.024 (0.036)	0.019 (0.028)	-0.270 ^a
CF-4	42	14.3	1.14	1.14	0.028 (0.026)	0.027 (0.025)	-0.037
CF-5	23	28.6	1.29	1.28	0.050 (0.041)	0.071 (0.050)	0.299 ^a
CF-6	18	4.8	1.05	1.05	0.019 (0.030)	0.018 (0.030)	-0.008
CF-7	76	9.5	1.10	1.10	0.044 (0.057)	0.044 (0.049)	0.001
CF-8	25	0.0	1.00	1.00	0.000 (0.000)	0.000 (0.000)	na
Average	38	11.9	1.12	1.11	0.031 (0.006)	0.034 (0.008)	0.030 ^b
Pooled samples	307	38.1	1.38		0.033 (0.018)	0.069 (0.034)	
Homosporous ferns ^c		36.1	1.63			0.132	

Abbreviations: n , sample size; % P , percentage of polymorphic loci; A , mean number of alleles per locus; AR , mean allelic richness based on a minimum sample size of 18 individuals; H_o , observed heterozygosity; H_e , Hardy-Weinberg (H-W) expected heterozygosity or gene diversity; SE, standard error; F_{IS} , fixation index within populations; na, not available (because of monomorphism across all the loci examined in this population).

^a Denotes significance ($P < 0.05$) based on permutation (999 replicates) under the null hypothesis of $F_{IS} = 0$.

^b Non-significant Weir and Cockerham (1984) estimate of F_{IS} over populations.

^c Allozyme-based genetic data from Tables 7 and 8 in Li and Haufler (1999).

populations. Alternatively, if extant populations were established from colonizers coming from a single source (i.e., a single refugium), then within-population genetic variation would be low because of long-distance dispersal associated bottlenecks (e.g., Hewitt, 1996, 2000). Genetic differentiation among populations would be high or low depending on rates of contemporary gene flow among the Korean populations. To date, these colonization hypotheses have not been empirically tested for the warm-temperate and subtropical homosporous ferns native to the Korean Peninsula. In this study, we surveyed the levels and distribution of allozyme-based genetic diversity in *C. falcatum* to test which of the post-glacial colonization hypotheses is most likely. Achieving a better understanding of the genetic structure of this currently rare fern in the Korean Peninsula, in addition, will provide guidelines for its recovery and management.

MATERIALS AND METHODS

Sample collection.—We collected one leaf segment (pinna) from each individual to minimize damage to the plants. A total of 307 individuals were sampled from eight populations of *C. falcatum* from southern Korea, including several islands (Fig. 1 and Table 1). All sampled leaf tissue was kept on ice until its transportation to the laboratory, where it was stored at 4°C until enzyme extraction.

Allozyme electrophoresis.—We extracted enzymes by finely cutting leaf samples, adding an extraction buffer (Mitton *et al.*, 1979), and then crushing them with a mortar and pestle. Enzyme extracts were absorbed onto chromatography wicks and stored in microtiter plates in an ultra-cold (-70°C) freezer until analyzed. We conducted electrophoresis on 13% starch gels, with three buffer systems. We used a modification (Haufler, 1985) of system 6 of Soltis *et al.* (1983) to resolve alcohol dehydrogenase (*Adh*), diaphorase (*Dia-1*, *Dia-2*), fluorescent esterase (*Fe-1*, *Fe-2*), and cathodal peroxidase (*Cpx*). We used system 11 of Soltis *et al.* (1983) to resolve glyceraldehyde-3-phosphate dehydrogenase (*G-3-pdh-1*, *G-3-pdh-2*), hexokinase (*Hk-1*, *Hk-2*), isocitrate dehydrogenase (*Idh*), phosphoglucosomerase (*Pgi-1*, *Pgi-2*), phosphoglucosomutase (*Pgm-1*, *Pgm-2*, *Pgm-3*), and shikimate dehydrogenase (*Skdh*). In addition, we used the morpholine-citrate buffer system (pH 6.1) of Clayton and Tretiak (1972) to resolve fructose-1,6-diphosphatase (*F1,6*) and malate dehydrogenase (*Mdh-1*, *Mdh-2*, *Mdh-3*). We followed stain recipes from Soltis *et al.* (1983) except for diaphorase (Cheliak and Pitel, 1984). We designated putative loci sequentially, with the most anodally migrating isozyme designated as 1, the next 2, and so on. We also designated different alleles within each locus sequentially by *a*, the next *b*, and so on. The observed enzyme banding patterns were consistent with their typical subunit structure and subcellular compartmentalization in diploid plants (Weeden and Wendel, 1989).

Data analysis.—We considered a locus to be polymorphic when two or more alleles were observed, regardless of their frequencies. We estimated the genetic diversity parameters within populations using the programs POPGENE (Yeh *et al.*, 1999) and FSTAT (Goudet, 1995): percent polymorphic loci ($\%P$), mean number of alleles per locus (*A*), allelic richness (*AR*) corrected by minimum sample size ($n = 18$ at CF-6, the population with the smallest sample size), observed heterozygosity (H_o), and Hardy-Weinberg (H-W) expected heterozygosity or Nei's (1978) gene diversity (H_e). Except for *AR* and H_o , these parameters were also estimated for the total samples as a whole (i.e., at the species level). To test for recent decreases in effective population size (bottlenecks), we evaluated differences across loci between the H-W H_e and the equilibrium heterozygosity (H_{eq}) expected assuming mutation-drift equilibrium. H-W H_e is not very sensitive to the fate of low frequency alleles, whereas H_{eq} is relatively sensitive to population bottlenecks, and declines as a result of the loss of such alleles. These differences ($H_e - H_{eq}$ calculated for a number of independent loci) were evaluated using a sign test and a Wilcoxon sign-rank test under an infinite allele model using the program BOTTLENECK (Piry *et al.*, 1999). Since allelic diversity is generally lost more rapidly than H_e (Nei *et al.*, 1975), recently bottlenecked populations will exhibit an excess of H-W H_e relative to H_{eq} (Cornuet and Luikart, 1996; Luikart *et al.*, 1998).

We used the program SPAGeDi (Hardy and Vekemans, 2002) to calculate population-level F_{IS} (inbreeding) and its significance level by 999 permutations under the null hypothesis of $F_{IS} = 0$. To measure deviations from H-W equilibrium at each polymorphic locus, we calculated averages of Wright's

TABLE 2. Results of statistical tests for evidence of recent population bottlenecks in *Cyrtomium falcatum*. Numbers reported are *P*-values of sign and Wilcoxon sign-rank tests conducted using the program BOTTLENECK.

Population	Sign test	Wilcoxon sign-rank test
CF-1	0.406	0.188
CF-2	0.442	0.188
CF-3	0.659	0.875
CF-4	0.605	0.938
CF-5	0.445	0.578
CF-6	0.519	0.250
CF-7	0.162	0.125
CF-8	na	na

(1965) F_{IS} and F_{ST} (deviations from H-W equilibrium of individuals relative to their local populations and local populations relative to the total population, respectively) following Weir and Cockerham (1984). Using FSTAT, we constructed 95% bootstrap confidence intervals (CI; 999 replicates) around means of F_{IS} and F_{ST} , and considered the observed F_{IS} and F_{ST} to be significant when the 95% CI did not overlap zero.

To test the overall pattern of genetic structure at the regional scale (i.e., isolation-by-distance effects), we conducted a Mantel test (Mantel, 1967) with 999 replicates, between all pairwise $F_{ST}/(1 - F_{ST})$ (F_{ST} was calculated following Weir and Cockerham, 1984) and the corresponding logarithm pairwise geographical distance (Rousset, 1997) under the null hypothesis of no spatial genetic structure (regression slope, $\beta = 0$). Finally, to determine the degree of genetic divergence among populations of *C. falcatum*, we calculated Nei's (1978) unbiased genetic identity (I) and distance (D) between pairs of populations. Using Nei's D values, we clustered populations into a phenogram following unweighted pair-group method using arithmetic averages (UPGMA).

RESULTS

Allozyme variation within populations.—Of the 21 putative loci resolved for *C. falcatum*, eight were polymorphic (*Dia-1*, *F1,6*, *Fe-1*, *Fe-2*, *Hk-2*, *Idh*, *Pgm-2*, and *Pgm-3*). Allozyme variation within populations was extremely low across the eight studied populations: mean percentage of polymorphic loci within populations ($\%P$) was 11.9, mean number of alleles per locus (A) was 1.12, and mean genetic diversity (H_e) was 0.034 (Table 1). Population CF-5 harbored the highest allelic richness and genetic diversity ($AR = 1.28$ and $H_e = 0.071$; Table 1), whereas no allozyme variation was found in CF-8 (Table 1). Slightly higher levels of genetic diversity were estimated from pooled samples over all populations ($n = 307$): $\%P = 38.1$; $A = 1.38$; and $H_e = 0.069$ (Table 1). Although we did not conduct any bottleneck test on CF-8 because it had no allozyme polymorphism, we found no significant indications of recent bottlenecks in any of the remaining seven populations (Table 2).

TABLE 3. Allele frequencies for the three loci with the highest degree of population differentiation (*F1,6*, *Pgm-2*, and *Pgm-3*).

Population	Allele frequency					
	<i>F1,6</i>		<i>Pgm-2</i>		<i>Pgm-3</i>	
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
CF-1	0.000	1.000	0.194	0.806	0.931	0.069
CF-2	0.000	1.000	0.514	0.486	0.292	0.708
CF-3	0.000	1.000	0.980	0.020	0.765	0.235
CF-4	0.000	1.000	0.083	0.917	0.083	0.917
CF-5	0.565	0.435	0.109	0.891	0.000	1.000
CF-6	0.750	0.250	1.000	0.000	0.000	1.000
CF-7	0.000	1.000	0.704	0.296	0.480	0.520
CF-8	1.000	0.000	0.000	1.000	0.000	1.000

Population genetic structure.—Except for CF-3 and CF-5, population-level F_{IS} estimates were not significantly different from zero at the 0.05 level (Table 1). These results, as well as the non-significant multi-population-level F_{IS} ($F_{IS} = 0.030$; Table 1 and 95% CI = -0.254 to 0.401), indicated that populations were generally at H-W equilibrium. Deviations from H-W expectations due to allele frequency differences between populations were, in contrast, significantly high ($F_{ST} = 0.543$, 95% CI = 0.218 to 0.703). This level of among-population differentiation was largely due to skewed allele frequencies at the three loci *F1,6*, *Pgm-2*, and *Pgm-3* (Table 3).

Pairwise Nei's (1978) I values between populations were high, ranging from 0.878 (CF-3 vs. CF-8) to 0.997 (CF-2 vs. CF-7) and with a mean of 0.951 ± 0.011 (SD), which is comparable with the average values reported for other conspecific populations of homosporous pteridophytes (average $I = 0.911 \pm 0.086$, $N = 16$; Soltis and Soltis, 1989) and of plants overall (average $I = 0.950 \pm 0.059$, $N = 1,572$; van der Bank *et al.*, 2001). The apparent discordance between the high values of F_{ST} and the high values of I in *C. falcatum* is simply due to the fact that only polymorphic loci are used for the calculation of F_{ST} , whereas both monomorphic and polymorphic loci are employed for estimating pairwise Nei's I . The UPGMA phenogram showed that the eight populations were clustered largely in accordance with their geographical locations: CF-1/CF-2 and CF-5/CF-6/CF-8 (which are located in the eastern and western extremes of southern part of Korea, respectively) were clustered separately (Fig. 2). However, we found no significant correlation between pairwise genetic differentiation estimates and their corresponding between-population logarithm pairwise geographical distance ($\beta = 0.069$, $R^2 = 0.016$, $P = 0.277$; Fig. 3), indicating that most variation (ca. 98%) in genetic differentiation was due to factors other than geographic distance.

DISCUSSION

Genetic diversity and structure.—Levels of within-population genetic diversity are extremely low in *C. falcatum* (mean population-level estimates;

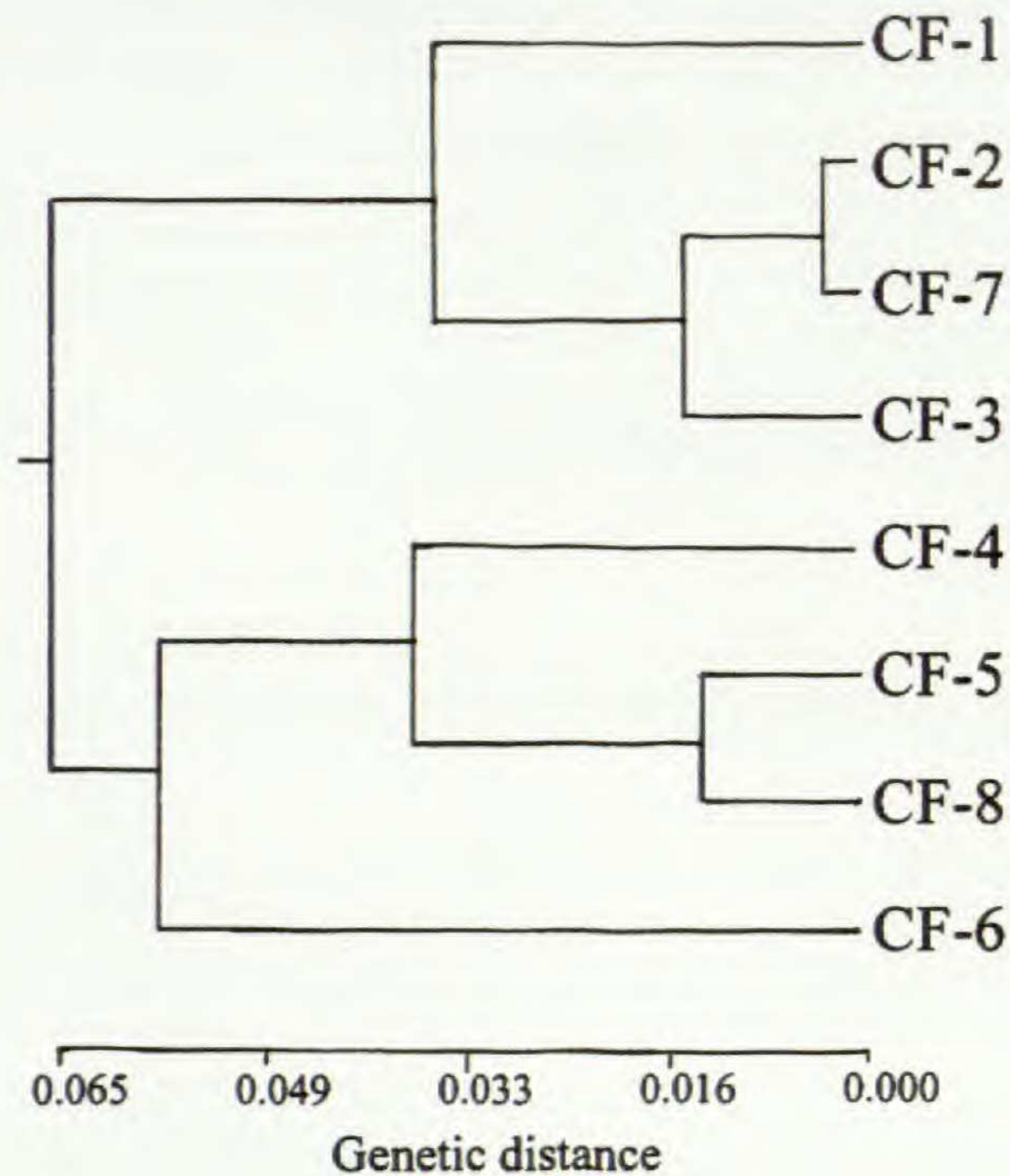


FIG. 2. UPGMA phenogram based on Nei's genetic distances between populations of *Cyrtomium falcatum*.

$%P = 11.9$, $A = 1.12$, $H_e = 0.034$). Although slightly higher values for these genetic diversity measures were obtained from pooled samples over all populations ($%P = 38.1$, $A = 1.38$, $H_e = 0.069$), A and H_e are still lower than expected for homosporous ferns (mean species-level estimates; $%P = 36.1$, $A = 1.63$, $H_e = 0.132$; Li and Haufler, 1999). The low levels of genetic variation in the southern Korean populations may be a consequence of post-glacial long-distance dispersal events and subsequent founder effects (see below for a detailed discussion).

Populations of *C. falcatum* were generally at H-W equilibrium (multi-population level $F_{IS} = 0.030$), a relatively unexpected finding since many homosporous ferns have potential for intragametophytic selfing (Klekowski and Baker, 1966), which could cause a substantial deviation from H-W equilibrium (i.e., a deficit of heterozygotes) within populations. Consistent with this expectation, a considerable excess of homozygotes has been found within populations of species of *Botrychium* and *Mankyua* (Ophioglossaceae), which have subterranean gametophytes that obligately self-fertilize via intragametophytic selfing (McCauley *et al.*, 1985; Soltis and Soltis, 1986; Watano and Sahashi, 1992; Hauk and Haufler, 1999; M. Y. Chung *et al.*, 2010). However, many diploid homosporous ferns exhibit high outbreeding rates (as inferred from inbreeding coefficients; Soltis and Soltis, 1989, 1992; Ranker and Geiger, 2008), and some studies have suggested that they possess mechanisms that promote outcrossing in natural populations (Klekowski, 1973; Haufler and Gastony, 1978; Haufler and Ranker, 1985; Wubs *et al.*, 2010). Some of these mechanisms promote the formation of functionally unisexual gametophytes

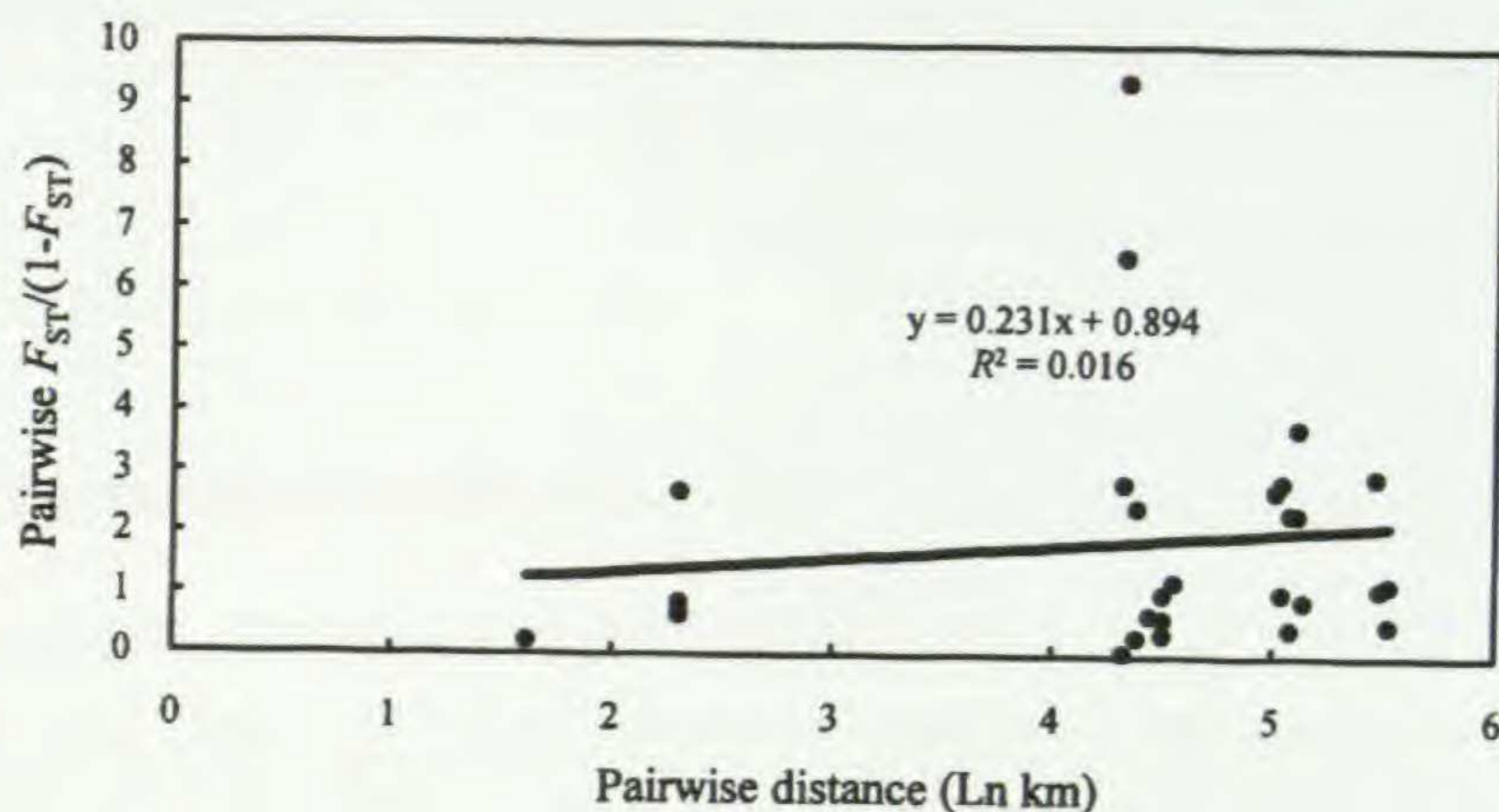


FIG. 3. Differentiation between populations of *Cyrtomium falcatum*. Multilocus estimates of pairwise differentiation of $F_{ST}/(1 - F_{ST})$ are plotted against pairwise logarithm (Ln) geographical distances in kilometers according to Rousset (1997). There was a non-significant positive relationship between pairwise $F_{ST}/(1 - F_{ST})$ and pairwise Ln geographical distance ($r = 0.126$, $P = 0.277$).

through the asynchronous maturation of male and female gametes and the control of antheridia initiation by the pheromone antheridiogen produced by maturing female gametophytes (Döpp, 1950; Lloyd, 1974; Haufler and Welling, 1994; Pajarón *et al.*, 1999). This seems to apply for populations of *C. falcatum*, although we do not know which of the above-mentioned mechanisms is promoting outcrossing in the Korean populations of this fern.

Outcrossing plant species usually maintain most of their genetic variation within rather than among populations, whereas selfing species show the reverse trend (Brown, 1979; Hamrick *et al.*, 1979). Thus, because populations of *C. falcatum* exhibit high inter-population divergence ($F_{ST} = 0.543$), factors other than mating system are probably important in shaping genetic structure among populations of *C. falcatum*. A high degree of genetic differentiation among populations has been observed in other homosporous ferns, including *Adiantum capillus-veneris* (Pryor *et al.*, 2001), *Asplenium csikii* (Vogel *et al.*, 1999b), *Asplenium ruta-muraria* (Schneller and Holderegger, 1996), *Asplenium septentrionale* (Holderegger and Schneller, 1994), *Asplenium trichomanes* subsp. *quadrivalens* (Suter *et al.*, 2000), *Cheilanthes gracillima* (Soltis *et al.*, 1989), and *Sadleria cyatheoides* and *S. pallida* (Ranker *et al.*, 1996). For all these cases, patchiness of suitable habitat (which caused restricted gene flow) has been proposed as a major driver of population divergence. This habitat trait may also account for the high among-population differentiation found in *C. falcatum* in southern Korea, which is primarily due to allele frequency differences at three loci (Table 3). For example, six of eight populations were monomorphic at *F1,6*; of these, the CF-8 population was fixed for the allele *a*, whereas the other five populations were fixed for the allele *b*. At *Pgm-2*, CF-6 was fixed for the allele *a*, whereas CF-8 was fixed for the alternative allele *b*. Apart from the low levels of gene flow, genetic drift would have been enhanced by small population sizes. Although current populations are of moderate size (M. Y. Chung and M. G. Chung, pers. observ.)

and we did not find any indications of recent bottlenecks (BOTTLENECK is only able to detect those bottlenecks that have occurred within approximately the past $2N_e$ – $4N_e$ generations; Piry *et al.*, 1999), the possibility of older bottlenecks should not be dismissed.

Inference of colonization history of C. falcatum in the southern Korean Peninsula.—Since *C. falcatum* is a member of the warm-temperate and subtropical vegetation community, and the Korean populations are at the northern edge of the species' geographic range, one may hypothesize that it endured the Quaternary glacial periods at more southerly latitudes. Glacial refugia for many elements of warm-temperate and subtropical flora have been proposed to occur in southern Kyushu and also in southern Honshu, Japan (see Fig. 1; Tsukada, 1984; Hattori, 1985; Matsuoka and Miyoshi, 1998; Aoki *et al.*, 2004; Gotanda and Yasuda, 2008). Fern spores usually have a high dispersal potential; ca. 500 ~ 800 km and even 3,200 km are suggested as maximum spore-dispersal distances (Tryon, 1970, 1972). The Tsushima (Korean) Strait was only about 10–20 km wide during the LGM and remained relatively narrow until ca. 14,000–12,000 yr BP (Park *et al.*, 2000; Lee *et al.*, 2008) being therefore easily passable. Even during the Holocene the 200 km channel width would have not constituted an insurmountable barrier for spore dispersal. Current populations of *C. falcatum* could also have arrived from the more distantly located southern regions, as the East China Sea (ECS) was largely exposed until at least 10,000 yr BP (Xu *et al.*, 2010). Therefore, migrations from southern China, Taiwan or even from some locations offshore in the southern part of the ECS cannot be ruled out (see Harrison *et al.*, 2001; Hope *et al.*, 2004; Prentice *et al.*, 2011).

The low within-population genetic variation for *C. falcatum* argues against the multiple-refugia hypothesis and supports the second hypothesis that the contemporary Korean populations of *C. falcatum* are descendant from colonizers from a single glacial refugium, presumably from southern Japan and/or southern China. However, we should bear in mind that these two proposed scenarios (multiple vs. single source populations) are the two extremes of a spectrum of possibility (e.g., some of the extant Korean populations could come from a single source, whereas others could originate from the admixture of several lineages). Moreover, many factors could have altered and/or modeled these “ideal” patterns, such as the number of colonization events, the number of propagules arriving at each colonization event, and the occurrence of genetic bottlenecks. For example, if population sizes have been historically small, random genetic drift since the post-glacial colonization events would have lead to low levels of intrapopulation genetic diversity even if the populations originated from multiple sources. In this latter case, patterns of genetic variation will be hardly distinguishable from those expected for species that immigrated from a single refugium. Clearly, more species (especially those continuously distributed) should be studied to draw firm conclusions about the post-glacial colonization history of warm-temperate homosporous fern populations currently occurring in Korea.

A similar scenario of glacial survival in remote refugia and post-glacial recolonization has been proposed for the homosporous fern *Dryopteris*

aemula. Jiménez *et al.* (2009) reported a total lack of allozyme variation ($H_T = 0.000$) of this fern in the Iberian Peninsula, which was attributed to founder effects during the Holocene expansion. Later, using five microsatellite loci and adding one population from the Macaronesian archipelago of Azores, Jiménez *et al.* (2010) found low levels of genetic variation within populations (total heterozygosity, $H_T = 0.447$) and a high degree of population genetic differentiation ($F_{ST} = 0.520$) in *D. aemula*. Interestingly, the Macaronesian population was much more variable than the Iberian ones and, based on these findings, the authors suggested that the Azores acted as a glacial refugium from which *D. aemula* spread northeastward and recolonized mainland Europe (Jiménez *et al.*, 2010). The role of glacial refugia as sources of plant diversity for the post-glacial recolonization in Europe of the Macaronesian Islands has been acknowledged in recent years (e.g., Caujapé-Castells, 2011; Fernández-Palacios *et al.*, 2011; Hutsemékers *et al.*, 2011).

In sum, southern Korean populations of *C. falcatum* exhibit low within-population genetic variation, which may be a consequence of post-glacial long-distance dispersal events, presumably from a single glacial refugium, and subsequent founder effects. In addition, restricted gene flow among the highly specific rock habitats on which *C. falcatum* occurs discontinuously in southern Korea would have contributed to the high degree of among-population genetic differentiation.

Conservation implications.—An understanding of how genetic diversity is partitioned within and among populations is critical to design adequate plant conservation plans (Godt and Hamrick, 2001; Sun and Wong, 2001). In order to preserve a representative sample of the genetic variation, species with high population differentiation require the conservation of more populations *in situ*, and also a more extensive population sampling for *ex situ* conservation. Since *C. falcatum* exhibits a high degree of divergence among populations, a relatively large number of populations should be targeted for both *in situ* and *ex situ* conservation. Using the formula proposed by Ceska *et al.* (1997), $P = 1 - (F_{ST})^n$ (where P is the proportion of genetic variation desired to be preserved and n is the number of populations to be sampled/protected), we should protect/sample at least four populations in order to conserve $\geq 90\%$ of the genetic diversity found in *C. falcatum*. Considering allelic richness, allele frequencies, and the UPGMA phenogram, we suggest that the populations CF-1 and CF-7 from one of the clusters and CF-5 and CF-6 from the other cluster deserve both *in situ* preservation and *ex situ* conservation in southern Korea. Thus, these populations should be protected by law (e.g., by designing plant reserves), whereas spores should be collected and deposited in spore storage facilities (e.g., by cryoconservation; Ballesteros *et al.*, 2012).

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LITERATURE CITED

- ADAMS, J. M. and H. FAURE. 1997. Review and atlas of palaeovegetation: preliminary land ecosystem maps of the world since the Last Glacial Maximum. Oak Ridge National Laboratory, Oak Ridge (TN). [cited 2012 May 12]. Available at: <http://www.esd.ornl.gov/projects/qen/adams1.html>.
- AOKI, K., T. SUZUKI, T.-W. HSU and N. MURAKAMI. 2004. Phylogeography of the component species of broad-leaved evergreen forests in Japan, based on chloroplast DNA variation. *J. Pl. Res.* 117:77–94.
- ARDITTI, J. and A. K. A. GHANI. 2000. Tansley review No. 110: Numerical and physical properties of orchid seeds and their biological implications. *New Phytol.* 145:367–421.
- BALLESTEROS, D., E. ESTRELLES, C. WALTERS and A. M. IBARS. 2012. Effects of temperature and desiccation on *ex situ* conservation of nongreen fern spores. *Amer. J. Bot.* 99:721–729.
- BROWN, A. H. D. 1979. Enzyme polymorphism in plant populations. *Theor. Populat. Biol.* 15:1–42.
- CAUJAPÉ-CASTELLS, J. 2011. Jesters, red queens, boomerangs and surfers: A molecular outlook on the diversity of the Canarian endemic flora, Pp. 284–324, *In*: D. Bramwell and J. Caujapé-Castells, eds. *The biology of island floras*. Cambridge University Press, London.
- CESKA, J. F., J. M. AFFOLTER and J. L. HAMRICK. 1997. Developing a sampling strategy for *Baptisia arachnifera* based on allozyme diversity. *Conservation Biol.* 11:1131–1139.
- CHELIAK, W. M. and J. P. PITEL. 1984. Technique for starch gel electrophoresis of enzyme from forest tree species. *Information report PI-X-42*. Petawawa National Forestry Institute, Chalk River, Ontario, Canada.
- CHOI, K.-R. 1998. The post-glacial vegetation history of the lowland in Korean Peninsula. *Korean J. Ecol.* 21:169–174.
- CHUNG, C.-H. 2007. Vegetation response to climate change on Jeju Island, South Korea, during the last deglaciation based on pollen record. *Geosci. J.* 11:147–155.
- CHUNG, C.-H. 2011. Holocene vegetation dynamics and its climatic implications inferred from pollen record in Boseong area, South Korea. *Geosci. J.* 15:257–264.
- CHUNG, C.-H., H. S. LIM and H. I. YOON. 2006. Vegetation and climate changes during the Late Pleistocene to Holocene inferred from pollen record in Jinju area, South Korea. *Geosci. J.* 10:423–431.
- CHUNG, C.-H., H. S. LIM and H. J. LEE. 2010. Vegetation and climate history during the late Pleistocene and early Holocene inferred from pollen record in Gwangju area, South Korea. *Quatern. Int.* 227:61–67.
- CHUNG, M. Y., J. D. NASON, B.-Y. SUN, M.-O. MOON, J. M. CHUNG, C.-W. PARK and M. G. CHUNG. 2010. Extremely low levels of genetic variation in the critically endangered monotypic fern genus *Mankyua chejuense* (Ophioglossaceae) from Korea: Implications for conservation. *Biochem. Syst. Ecol.* 38:888–896.
- CLAYTON, J. W. and D. N. TRETIAK. 1972. Amine citrate buffers for pH control in starch gel electrophoresis. *J. Fish. Res. Board Canada* 29:1169–1172.
- CORNUET, J. M. and G. LUIKART. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144:2001–2014.
- DÖPP, W. 1950. Eine die Antheridienbildung bei farnen fördernde substanz in den prothallien von *Pteridium aquilinum* (L.) Kuhn. *Ber. Deutsch. Bot. Ges.* 63:139–147.
- DUMINIL, J., S. FINESCHI, A. HAMPE, P. JORDANO, D. SALVINI, G. G. VENDRAMIN and R. J. PETIT. 2007. Can population genetic structure be predicted from life-history traits? *Amer. Naturalist* 169:662–672.
- EDGINTON, J. A. 2007. Dynamics of long-distance dispersal: the spread of *Asplenium adiantum-nigrum* and *Asplenium trichomanes* (Aspleniaceae; Pteridophyta) on London walls. *Fern Gaz.* 18:31–38.
- FERNÁNDEZ-PALACIOS, J. M., L. DE NASCIMENTO, R. OTTO, J. D. DELGADO, E. GARCÍA-DEL-REY, J. R. ARÉVALO and R. J. WHITTAKER. 2011. A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forests. *J. Biogeogr.* 38:226–246.
- FLINN, K. M. 2006. Reproductive biology of three fern species may contribute to differential colonization success in post-agricultural forests. *Amer. J. Bot.* 93:1289–1294.

- GODT, M. J. W. and J. L. HAMRICK. 2001. Genetic diversity in rare southeastern plants. *Nat. Areas J.* 21:61–70.
- GOTANDA, K. and Y. YASUDA. 2008. Spatial biome changes in southwestern Japan since the Last Glacial Maximum. *Quatern. Int.* 184:84–93.
- GOUDET, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. *J. Heredity* 86:485–486.
- GRAY, A. 1996. Genetic diversity and its conservation in natural populations of plants. *Biodivers. Lett.* 3:71–80.
- HAMRICK, J. L. and M. J. W. GODT. 1989. Allozyme diversity in plant species, Pp. 43–63, *In*: A. H. D. Brown, M. T. Clegg, A. L. Kahler and B. S. Weir, eds. *Plant population genetics, breeding and genetic resources*. Sinauer Associates, Sunderland.
- HAMRICK, J. L. and J. D. NASON. 1996. Consequences of dispersal in plants, Pp. 203–236, *In*: O. E. Rhodess Jr, R. K. Chesser and M. H. Smith, eds. *Population dynamics in ecological space and time*. University of Chicago Press, Chicago.
- HAMRICK, J. L., Y. B. LINHART and J. B. MITTON. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Rev. Ecol. Syst.* 10:173–200.
- HARDY, O. J. and X. VEKEMANS. 2002. SPAGeDi: a versatile computer program to analyze spatial genetic structure at the individual or population levels. *Molec. Ecol. Notes* 2:618–620.
- HARRISON, S. P., G. YU, H. TAKAHARA and I. C. PRENTICE. 2001. Diversity of temperate plants in east Asia. *Nature* 413:129–130.
- HATTORI, T. 1985. Synecological study on the lucidophyllous forest of *Castanopsis-Persea* type in Japan proper. *Bull. Kobe. Geobot. Soc.* 1:1–98 (in Japanese with English abstract).
- HAUFLER, C. H. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). *Syst. Bot.* 10:92–104.
- HAUFLER, C. H. and G. J. GASTONY. 1978. Antheridiogen and the breeding system in the fern genus *Bommeria*. *Canad. J. Bot.* 56:1594–1601.
- HAUFLER, C. H. and T. A. RANKER. 1985. Effects of differential antheridiogen response on evolutionary mechanisms in *Cystopteris*. *Amer. J. Bot.* 71:878–881.
- HAUFLER, C. H. and C. B. WELLING. 1994. Antheridiogen, dark spore germination, and outcrossing mechanisms in *Bommeria* (Adiantaceae). *Amer. J. Bot.* 81:616–621.
- HAUK, W. D. and C. H. HAUFLER. 1999. Isozyme variability among crytic species of *Botrychium* subgenus *Botrychium* (Ophioglossaceae). *Amer. J. Bot.* 86:614–633.
- HEWITT, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* 58:247–276.
- HEWITT, G. M. 1999. Post-glacial re-colonization of European biota. *Biol. J. Linn. Soc.* 68:87–112.
- HEWITT, G. M. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405:907–913.
- HEWITT, G. M. 2011. Quaternary phylogeography: the roots of hybrid zones. *Genetica* 139:617–638.
- HOLDEREGGER, R. and J. J. SCHNELLER. 1994. Are small isolated populations of *Asplenium septentrionale* variable? *Biol. J. Linn. Soc.* 51:377–385.
- HOPE, G., A. P. KERSHAW, S. VAN DER KAARS, X. SUN, P.-M. LIEW, L. E. HEUSSER, H. TAKAHARA, M. MCGLONE, N. MIYOSHI and P. T. MOSS. 2004. History of vegetation and habitat change in the Austral-Asian region. *Quatern. Int.* 118–119:103–126.
- HU, F. S., A. HAMPE and R. J. PETTIT. 2009. Paleoecology meets genetics: deciphering past vegetational dynamics. *Frontiers Ecol. Environ.* 7:371–379.
- HUTSEMÉKERS, V., P. SZÖVÉNYI, A. J. SHAW, J.-M. GONZÁLEZ-MANCEBO, J. MUÑOZ and A. VANDERPOORTEN. 2011. Oceanic islands are not sinks of biodiversity in spore-producing plants. *Proc. Natl. Acad. Sci. USA* 108:18989–18994.
- IWATSUKI, K. 1992. *Ferns and fern allies of Japan*. Heibonsha Ltd., Tokyo, Japan.
- JIMÉNEZ, A., L. G. QUINTANILLA, S. PAJARÓN and E. PANGUA. 2009. Genetic variation in the allotetraploid *Dryopteris corleyi* and its diploid parental species in the Iberian Peninsula. *Amer. J. Bot.* 96:1880–1886.
- JIMÉNEZ, A., R. HOLDEREGGER, D. CSENCICS and L. G. QUINTANILLA. 2010. Microsatellites reveal substantial among-population genetic differentiation and strong inbreeding in the relict fern *Dryopteris aemula*. *Ann. Bot.* 106:149–155.

- KLEKOWSKI, E. J. 1972. Genetical features of ferns as contrasted to seed plants. *Ann. Missouri Bot. Gard.* 59:138–151.
- KLEKOWSKI, E. J. 1973. Sexual and subsexual systems in the homosporous ferns: a new hypothesis. *Amer. J. Bot.* 60:535–544.
- KLEKOWSKI, E. J. and H. G. BAKER. 1966. Evolutionary significance of polyploidy in the Pteridophyta. *Science* 153:305–307.
- LEE, E., S. KIM and S. NAM. 2008. Paleo-Tsushima Water and its effect on surface water properties in the East Sea during the last glacial maximum: Revisited. *Quatern. Int.* 176–177:3–12.
- LI, J. and C. H. HAUFLE. 1999. Genetic variation, breeding systems, and patterns of diversification in Hawaiian *Polypodium* (Polypodiaceae). *Syst. Bot.* 24:339–355.
- LLOYD, R. M. 1974. Reproductive biology and evolution in the Pteridophyta. *Ann. Missouri Bot. Gard.* 61:318–331.
- LÓPEZ-PUJOL, J., F.-M. ZHANG, H.-Q. SUN, T.-S. YING and S. GE. 2011. Centres of plant endemism in China: places for survival or for speciation? *J. Biogeogr.* 38:1267–1280.
- LUIKART, G., F. W. ALLENDORF, J. M. CORNUET and W. B. SHERWIN. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Heredity* 89:238–247.
- MANTEL, N. A. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27:209–220.
- MATSUOKA, K. and N. MIYOSHI. 1998. Chapter III-4, Pp. 224–236, *In: Y. Yasuda and N. Miyoshi, eds. The illustrated vegetation history of the Japanese Archipelago.* Asakura-shoten, Toyko, Japan (in Japanese).
- MCCAULEY, D. E., D. P. WHITTIER and L. M. REILLY. 1985. Inbreeding and the rate of self-fertilization in a grape fern, *Botrychium dissectum*. *Amer. J. Bot.* 72:1978–1981.
- MITTON, J. B., Y. B. LINHART, K. B. STURGEON and J. L. HAMRICK. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *J. Heredity* 70:86–89.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.
- NEI, M., T. MARUYAMA and R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. *Evolution* 29:1–10.
- NEVO, E., A. BEILES and R. BEN-SHLOMO. 1984. The evolutionary significance of genetic diversity: Ecological, demographic and life history correlates, Pp. 13–213, *In: G. S. Mani, ed. Evolutionary dynamics of genetic diversity – Lecture Notes in Biomathematics (vol. 53).* Springer-Verlag, Berlin.
- PAJARÓN, S., E. PANGUA and L. GARCÍA-ÁLVAREZ. 1999. Sexual expression and genetic diversity in populations of *Cryptogramma crispa* (Pteridaceae). *Amer. J. Bot.* 86:964–973.
- PARK, S.-C., D.-G. YOO, C.-W. LEE and E.-I. LEE. 2000. Last-glacial sea level changes and paleogeography of the Korea (Tsushima) Strait. *Geo-Mar. Lett.* 20:64–71.
- PECK, J. H., C. J. PECK and D. R. FARRAR. 1990. Influences of life history attributes on formation of local and distant fern populations. *Amer. Fern J.* 80:126–142.
- PETT, R. J., I. AGUINAGALDE, J. L. DE BEAULIEU, C. BITTKAU, S. BREWER, R. CHEDDADI, R. ENNOS, S. FINESCHI, D. GRIVET, M. LASCoux, A. MOHANTY, G. MUELLER-STARCK, B. DEMESURE-MUSCH, A. PALME, J. P. MARTIN, S. RENDELL and G. G. VENDRAMIN. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* 300:1563–1565.
- PIRY, S., G. LUIKART and J.-M. CORNUET. 1999. Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Heredity* 90:502–503.
- PRENTICE, I. C., S. P. HARRISON and P. J. BARTLEIN. 2011. Global vegetation and terrestrial carbon cycle changes after the last ice age. *New Phytol.* 189:988–998.
- PRYOR, K. V., J. E. YOUNG, F. J. RUMSEY, K. J. EDWARDS, M. W. BRUFORD and H. J. ROGERS. 2001. Diversity, genetic structure and evidence of outcrossing in British populations of the rock fern *Adiantum capillus-veneris* using microsatellites. *Molec. Ecol.* 10:1881–1894.
- QIU, Y.-X., C.-X. FU and H. P. COMES. 2011. Plant molecular phylogeography in China and adjacent regions: Tracing the genetic imprints of Quaternary climate and environmental change in the world's most diverse temperate flora. *Molec. Phylogen. Evol.* 59:225–244.

- RANKER, T. A. and J. M. O. GEIGER. 2008. Population genetics, Pp. 175–198, *In*: R. A. Ranker and C. H. Haufler, eds. *Biology and evolution of ferns and lycophytes*. Cambridge University Press, Cambridge.
- RANKER, T. A., C. E. C. GEMMILL, P. G. TRAPP, A. HAMBELTON and K. HA. 1996. Population genetics and reproductive biology of lava-flow colonising species of Hawaiian *Sadleria* (Blechnaceae), Pp. 581–598, *In*: J. M. Camus, M. Gibby and R. J. Johns, eds. *Pteridophytes in perspective*. Royal Botanic Gardens, Kew.
- ROUSSET, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* 145:1219–1228.
- ROUX, J. P. 2011. The genus *Cyrtomium* (Pteridophyta: Dryopteridaceae) in Africa and Madagascar. *Bot. J. Linn. Soc.* 167:449–465.
- SCHNELLER, J. J. and R. HOLDEREGGER. 1996. Colonisation events and genetic variability within populations of *Asplenium ruta-muraria* L., Pp. 571–580, *In*: J. M. Camus, M. Gibby and R. J. Johns, eds. *Pteridophytes in perspective*. Royal Botanic Gardens, Kew.
- SHI, Y. 2002. Characteristics of late Quaternary monsoonal glaciation on the Tibetan Plateau and in East Asia. *Quatern. Int.* 97–98:79–91.
- SOLTIS, D. E. and P. S. SOLTIS. 1986. Electrophoretic evidence for inbreeding in the fern *Botrychium virginianum* (Ophioglossaceae). *Amer. J. Bot.* 73:588–592.
- SOLTIS, D. E. and P. S. SOLTIS. 1989. Polyploidy, breeding systems, and genetic differentiation in homosporous Pteridophytes. Pp. 241–258, *In*: D. E. Soltis and P. S. Soltis, eds. *Isozymes in plant biology*. Dioscorides Press, Portland.
- SOLTIS, D. E. and P. S. SOLTIS. 1992. The distribution of selfing rates in homosporous ferns. *Amer. J. Bot.* 79:97–100.
- SOLTIS, D. E., C. H. HAUFLE, D. C. DARROW and G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Amer. Fern J.* 73:9–27.
- SOLTIS, D. E., A. B. MORRIS, J. S. MCLACHLAN and P. S. MANOS. 2006. Comparative phylogeography of unglaciated eastern North America. *Molec. Ecol.* 15:4261–4293.
- SOLTIS, P. S., D. E. SOLTIS and B. D. NESS. 1989. Population genetic structure in *Cheilanthes gracillima*. *Amer. J. Bot.* 76:1114–1118.
- SUN, M. and K. C. WONG. 2001. Genetic structure of three orchid species with contrasting breeding systems using RAPD and allozyme markers. *Amer. J. Bot.* 88:2180–2188.
- SUTER, M., J. J. SCHNELLER and J. C. VOGEL. 2000. Investigations into the genetic variation, population structure and breeding system of the fern *Asplenium trichomanes* subsp. *quadrivalens*. *Int. J. Pl. Sci.* 161:233–244.
- TRYON, R. 1970. Development and evolution of fern floras of oceanic islands. *Biotropica* 2:76–84.
- TRYON, R. 1972. Endemic areas and geographic speciation in tropical American ferns. *Biotropica* 4:121–131.
- TSUKADA, M. 1984. A vegetation map in the Japanese Archipelago approximately 20,000 years B.P. *Jap. J. Ecol.* 34:203–208 (in Japanese with English abstract).
- VAN DER BANK, H., M. VAN DER BANK and B.-E. VAN WYK. 2001. A review of the use of allozyme electrophoresis in plant systematics. *Biochem. Syst. Ecol.* 29:469–483.
- VOGEL, J. C., F. J. RUMSEY, J. J. SCHNELLER, J. A. BARRETT and M. GIBBY. 1999a. Where are the glacial refugia in Europe? Evidence from pteridophytes. *Biol. J. Linn. Soc.* 66:23–37.
- VOGEL, J. C., F. J. RUMSEY, S. J. RUSSELL, C. J. COX, J. S. HOLMES, W. BUJNOCH, C. STARK, J. A. BARRETT and M. GIBBY. 1999b. Genetic structure, reproductive biology and ecology of isolated populations of *Asplenium csikii* (Aspleniaceae, Pteridophyta). *Heredity* 83:604–612.
- WATANO, Y. and N. SAHASHI. 1992. Predominant inbreeding and its genetic consequences in a homosporous fern genus, *Sceptridium* (Ophioglossaceae). *Syst. Bot.* 17:486–502.
- WEEDEN, N. F. and J. F. WENDEL. 1989. Genetics of plant isozymes. Pp. 46–72, *In*: D. E. Soltis and P. S. Soltis, eds. *Isozymes in plant biology*. Dioscorides Press, Portland.
- WEIR, B. S. and C. C. COCKERHAM. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38:1358–1370.
- WEISS, S. and N. FERRAND, (eds.) 2007. *Phylogeography of southern European refugia – Evolutionary perspectives on the origins and conservation of European biodiversity*. Springer, Dordrecht.

- WIDMER, A. and C. LEXER. 2001. Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. *Trends Ecol. Evol.* 16:267–269.
- WRIGHT, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19:395–420.
- WUBS, E. R., G. A. DE GROOT, H. J. DURING, J. C. VOGEL, M. GRUNDMANN, P. BREMER and H. SCHNEIDER. 2010. Mixed mating system in the fern *Asplenium scolopendrium*: implications for colonization potential. *Ann. Bot.* 106:583–590.
- XU, D., H. LU, N. WU and Z. LIU. 2010. 30 000-Year vegetation and climate change around the East China Sea shelf inferred from a high-resolution pollen record. *Quatern. Int.* 227:53–60.
- YEH, F. C., YANG, R.-C. and T. B. J. BOYLE. 1999. POPGENE version 1.31-Microsoft Windows-based freeware for population genetic analysis. Quick users' guide. University of Alberta, Edmonton.
- YI, S. 2011. Holocene vegetation responses to East Asian monsoonal changes in South Korea. Pp. 157–178, *In*: J. Blanco and H. Kheradmand, eds. *Climate change–geophysical foundations and ecological effects*. In Tech, Rijeka (Croatia).
- YI, S. and S.-J. KIM. 2010. Vegetation changes in western central region of Korean Peninsula during the last glacial (ca. 21.1–26.1 cal kyr BP). *Geosci. J.* 14:1–10.